See also "Gypsy Moth Egg Mass Hatch Potential Methodology" by George Saufley

# Northeastern Area State & Private Forestry

P-72-2 5230

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## A METHOD FOR CLEANING, COUNTING AND SORTING

FEBRUARY 1972

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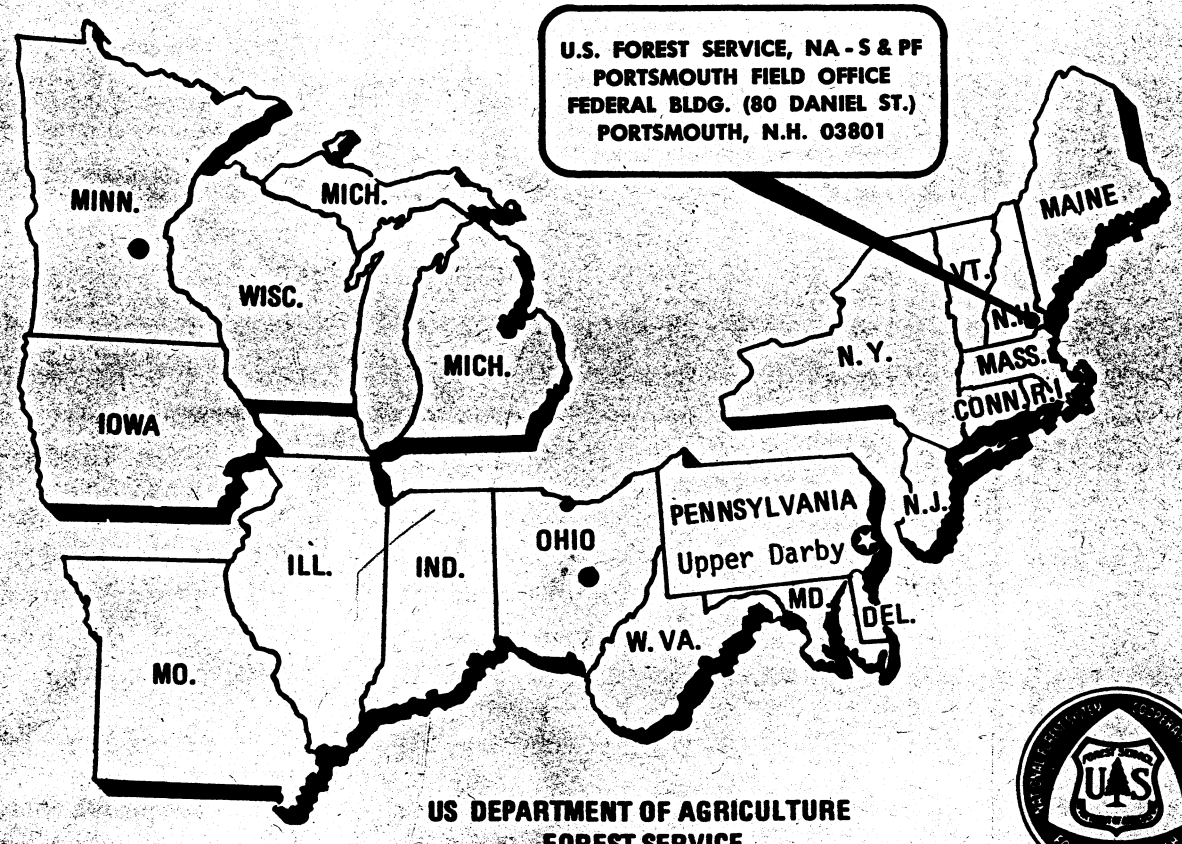


US DEPARTMENT OF AGRICULTURE
FOREST SERVICE

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## A Method for Cleaning, Counting and Sorting

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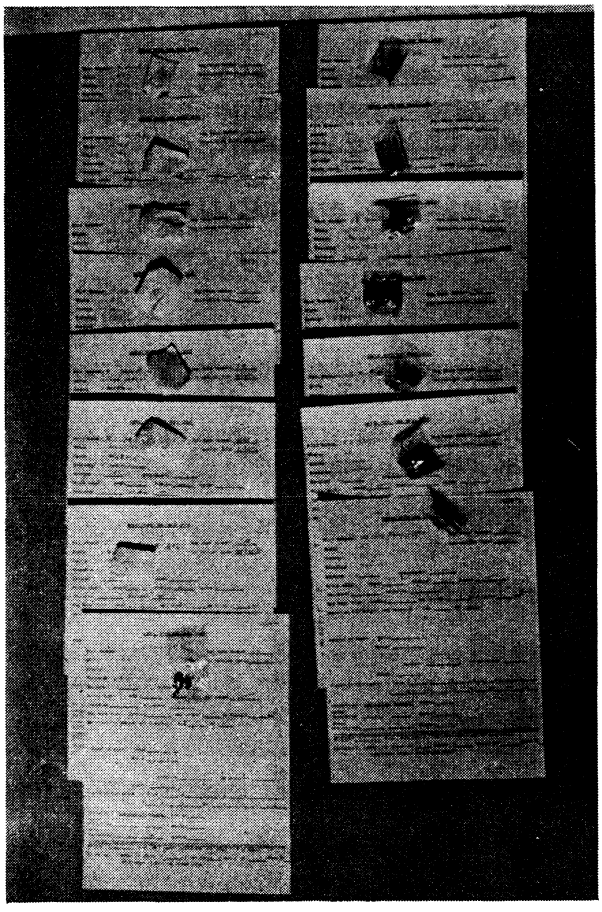


Photograph 1
Gypsy Moth Egg masses for processing
with corresponding data sheets.

INTRODUCTION

In 1971 a plan was developed to evaluate the effectiveness of the 1971 suppression projects against the gypsy moth in New Jersey, New York and Pennsylvania. A supplement to the work plan, October 1971, described the procedures for collecting and processing gypsy moth egg masses. This report describes the equipment and methods for "de-hairing", counting, and sorting the eggs referred to on pages 3 and 4 of the work plan supplement.

OBJECTIVES

1. To remove the major portion of "hair" that surrounds the eggs in a gypsy moth egg mass.

2. To count the number of eggs in individual egg masses by a rapid method.

3. To sort the eggs into three major categories:

   a. viable eggs

   b. parasitized eggs

   c. aborted eggs

METHODS AND MATERIALS

1. "De-hairing" and sorting apparatus and use of our de-hairing machinery was patterend after Pete Minor's device used in New Jersey. It consisted of a vacuum cleaner and a home-made attachment as shown in photograph 2. The vacuum cleaner was a Sears* model 11621891 canister type with flexible hose. (Figure 1A) The attachment consisted of two ordinary "tin" cans, made up in the following manner:

   a. A 1¼ hole was made through the center of the bottom of can "A". A 1¼" diameter brass tube 2" long was soldered against the hole in the can to make a funnel-like

* Use of a brand name does not imply endorsement of a product.

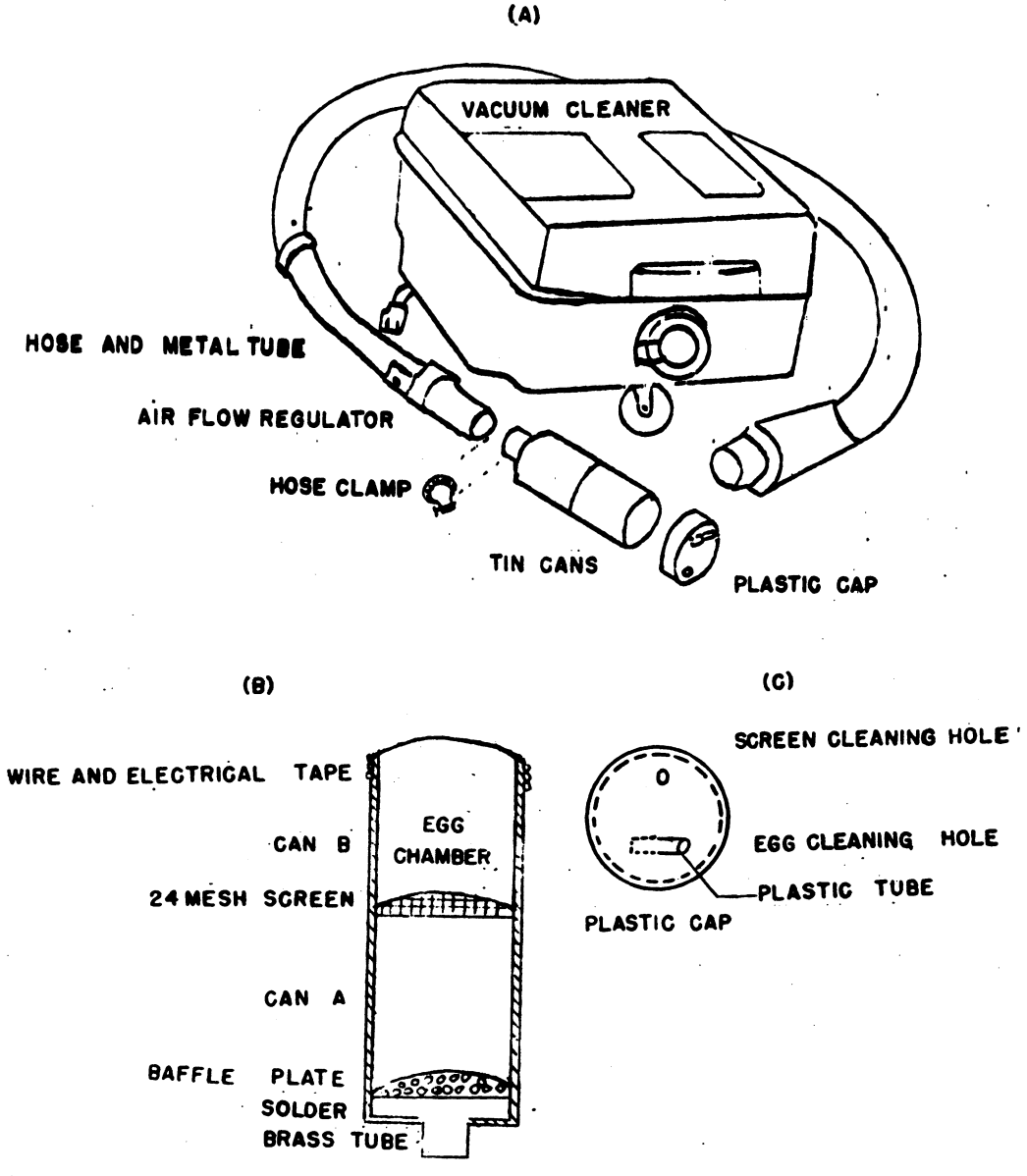



Figure 1. Apparatus for "de-hairing" gypsy moth egg masses. (A) Vacuum cleaner with tin can attachment. (B) Detail of tin can attachment. (C) Cap for egg chamber of tin can attachment.

arrangement. It was necessary to coat the entire bottom of the can with solder to insure stability. The outer coating was removed from places on the can where solder was applied.

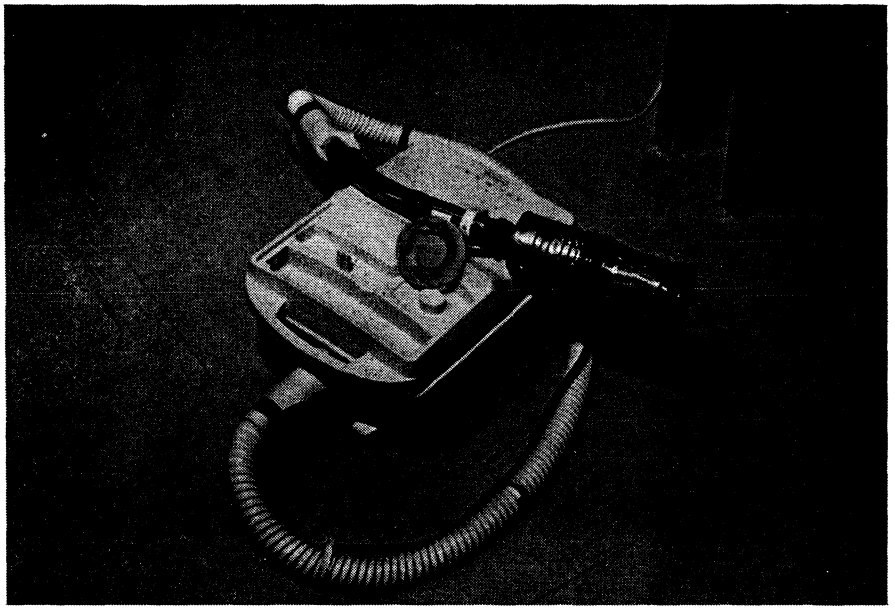
b. A baffle plate was cut to the diameter of can "A" then fastened with silicone cement inside the can ½" from, and parallel to the bottom of the can. The plate contained 300 evenly distributed holes, each 1/16" in diameter. This baffle plate provided better distribution of air currents within the can.

c. Can "B" had top and bottom removed and the bottom replaced with a 24 mesh screen affixed with silicone cement. The top lid of can "B" was protected against wear and tear by three turns of bare number 12 wire covered with four turns of electrical tape wrapped around the outside.

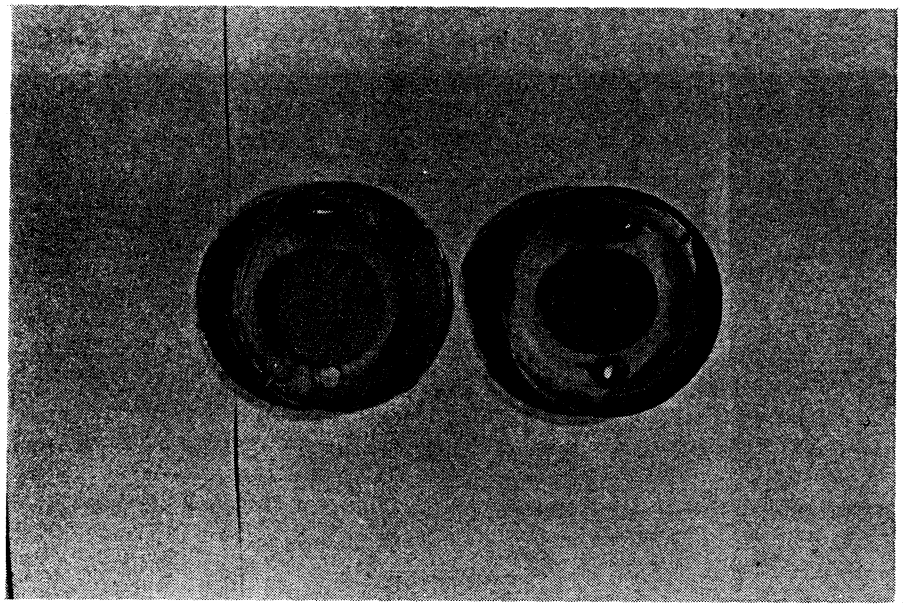
d. The bottom opening of Can "B" was soldered and taped to the top opening of can "A" to form a cylinder as shown in Figure 1B.

e. The brass tube on the bottom of can "A" was slipped over the attachment end of the vacuum cleaner hose and held in place with a hose clamp. The clamp was wrapped with electrical tape to make a smooth grip. It was necessary to cut and flare the brass tube for a good fit over the vacuum hose.

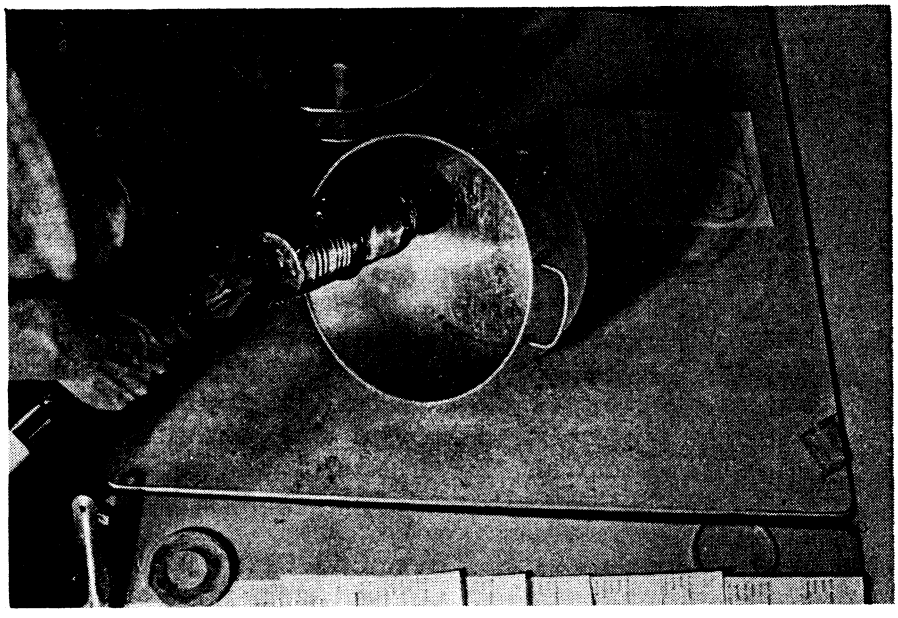
f. The bottom of a plastic petri dish was inverted and epoxy-glued to the inside of the petri dish top. This plastic lid was placed over the top of can "B" to prevent escape of gypsy moth eggs as they bounced around in can "B" during "de-hairing". Air was admitted through two 1/3" holes drilled through the plastic lid. A ½" piece of ¼" plastic tubing was glued into one hole at a $30^{o}$ angle in order to direct air flows around the edge of the can. Each hole was put near the edge of the lid. (Figure 1C, Photograph 3)



Photograph 2
Dehairing and sorting machine made from vacuum cleaner.



Photograph 3
Top and bottom view of plastic cap used for cleaning and dehairing eggs.



Photograph 4
Sorting and dehairing gypsy moth eggs by tapping the tin can device on the side of an aluminum kettle.

The "de-hairing" apparatus was operated in the following manner:

a. The egg cluster was placed in Can "B", and the plastic lid was put on.

b. The vacuum cleaner was turned on with its adjustable suction control valve on the hose in the ½ closed position.

c. The plastic lid was rotated 3 to 4 times while the eggs bounced about in Can "B" and the hairs were sucked through the screen into the vacuum cleaner.

d. When the operator saw that the hair was cleared away, he opened the suction control valve, inverted Can "B" in a four-quart aluminum kettle, removed the plastic lid, then tapped Can "B" against the inside of the kettle to collect the viable eggs.

e. The operator next inverted Can "B" in another four-quart kettle, turned off the vacuum cleaner, then tapped Can "B" against the inside of the kettle to collect parasitised and aborted eggs. (Photograph 4)

It required about 30 seconds to de-hair and sort the eggs into the two groups of viable and others plus bark flakes. The counting device described next was used to separate eggs from trash.

2. Egg Counting Equipment and Use

A pipette graduated in hundreds of milliliters was used to count eggs volumetrically. A sample of 720 viable, de-haired eggs was put into the Kimble disposable pipette. The 720 egg count was then divided by the volume in milliliters to arrive at a mean number of eggs per 0.01 ml. Values for other volumes were calculated by cumulation of the mean, and arranged in a number to volume ratio chart. Appendix A shows egg counts for various volumes of viable eggs and Appendix B shows counts for

other eggs. The reliability of the charts was tested during the first week of use by random selection of 26 samples. Chart values and actual counts were subjected to a paired t test. There was no significant difference at the 95% level. Calculations are shown in Appendix C.

The egg counter consisted of a pipette, a siphon valve, a plastic tube, and a vacuum pump arranged as in Figure 2. The pump was a model 500 58 AF 713 fitted with a four foot length of ¼-inch poly propylene tubing. (Photograph 5) The siphon valve (S.P. TRU Flate) was attached to the free end of the tube for easy control of vacuum. The pipette was fitted at the base with a ½-inch length of plastic tubing to insure an air-tight joint with the siphon valve. A piece of 24 guage copper screening was coiled and placed in the pipette at the zero mark to prevent loss of eggs into the vacuum pump.

The counting of eggs immediately followed the de-hairing and sorting procedures. The pipette, with vacuum pump on, was moved about in the kettles described above in order to pick up all eggs while avoiding bark flakes. (Photograph 6)

When all eggs in a kettle were picked up by the pipette, the volume was read and the number of eggs determined from the appropriate chart. (Appendix A and B) (Photograph 7) The number was recorded on the data sheet. (Appendix D) Plastic 10 dram vials were used to receive the eggs from the pipette by putting the tip of the pipette into the vial, releasing the vacuum, and tapping the pipette against the vial.

3. Separation of Parasitised, Hatched and Aborted Eggs

Following the sorting of eggs into two major groups of viable and others, it was found that some viable eggs were being held by the vacuum

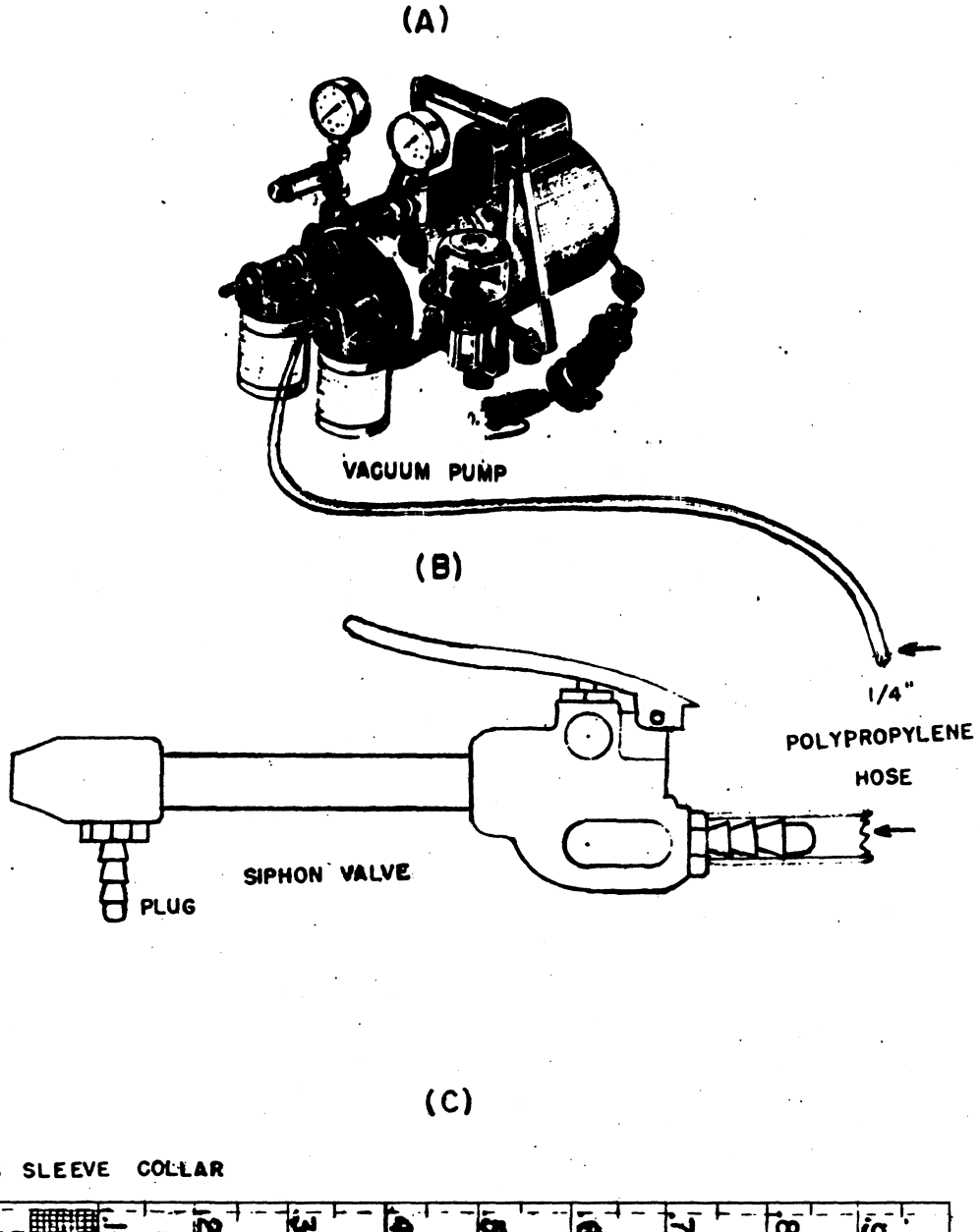
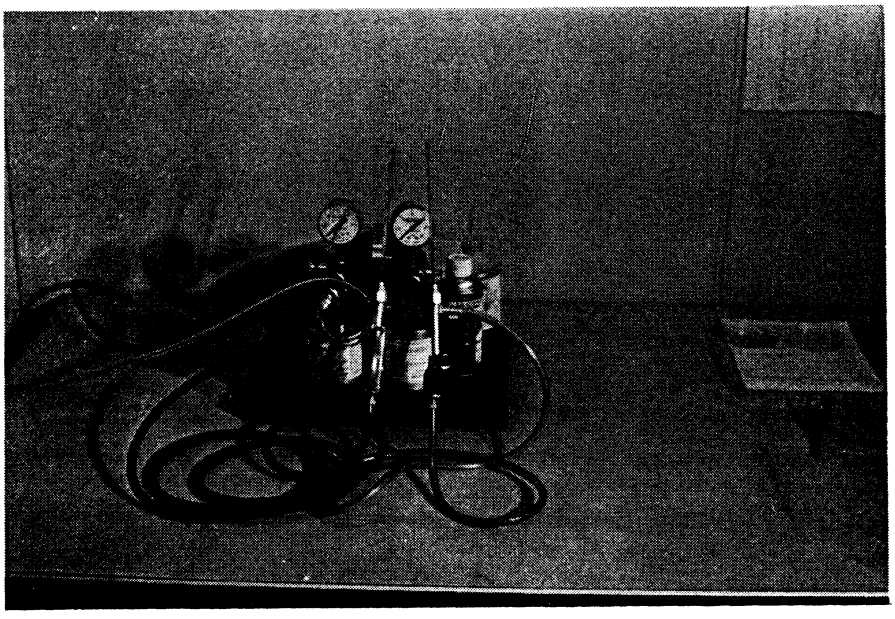
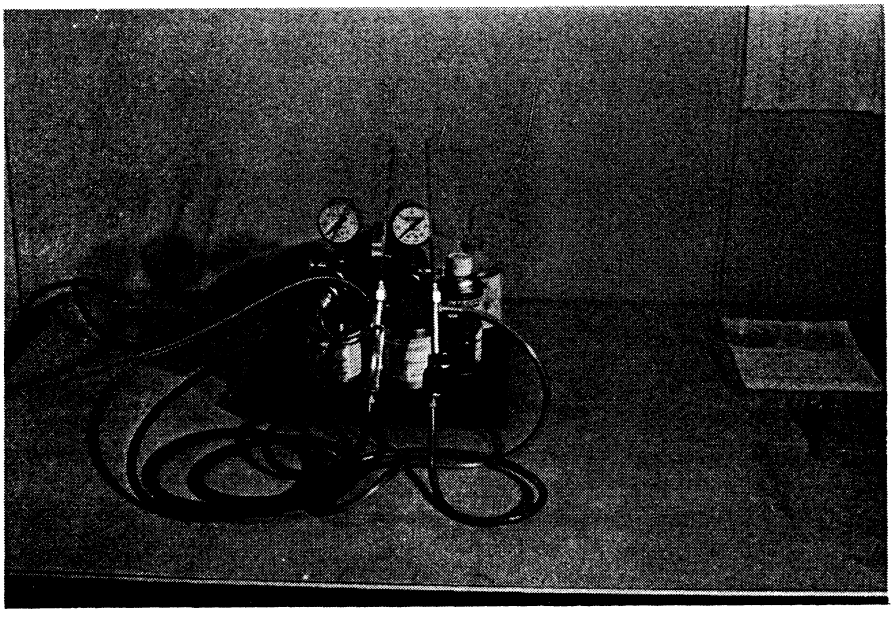


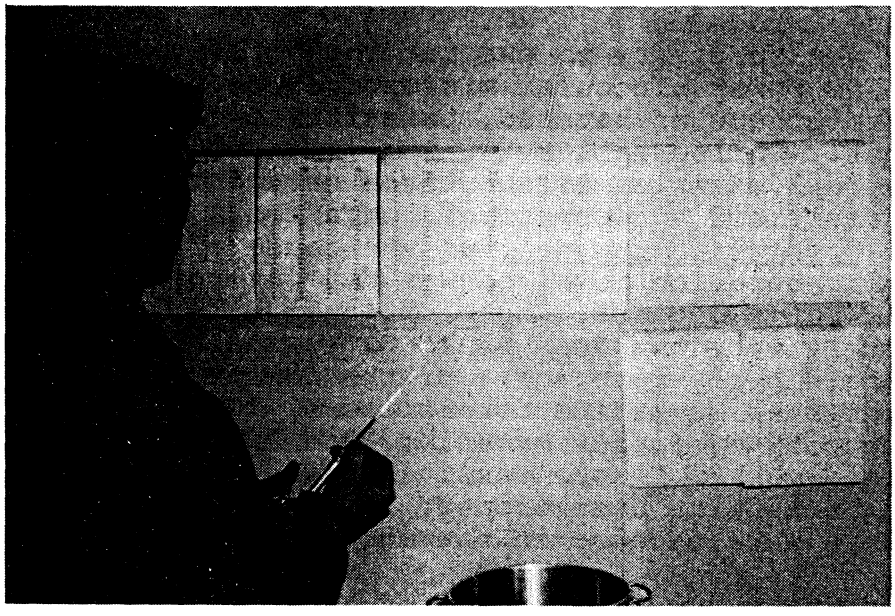

Figure 2. Gypsy moth egg counter (A) vacuum pump (B) siphon valve (C) pipette with plastic collar and wire mesh egg stop.



Photograph 5
Vacuum pump used to count gypsy moth eggs and sort out bark scraps.



Photograph 5
Vacuum pump used to count gypsy moth eggs and sort out bark scraps.



Photograph 7
Forestry aid counting eggs using the pipette and number to volume ratio charts.

cleaner attachment so that they were placed in the vial with aborted and parasitised eggs. While this did not interfere with the job at hand, it could mean loss of some viable eggs in later work. A Chi Square Test was applied on 90 samples to test for a significant loss of viable eggs to the "other" classification. There was no significant difference at the 95% level as shown in Appendix E.

It was necessary to examine the "other" under a 150 power binocular microscope for separation into groups of hatched, parasitised and aborted eggs. Photographs 8, 9, 10, 11 and 12 shows typical examples of the three categories. A parasitised egg sometimes contained a cadaver of Ooencyctus kuwanai, but the cause of mortality and generation involved was not determined. In all but two cases, the O. kuwanai were adults. Rarely were two dead adult O. kuwanai found in a single gypsy moth egg. Of the aborted eggs, 80% contained mummified caterpillars, 10% were occupied by fungus mycelium, and 10% by undeveloped yolk. In one case, all eggs within the egg mass were undeveloped; and in another case, there were no eggs in the egg mass.

CONCLUSIONS

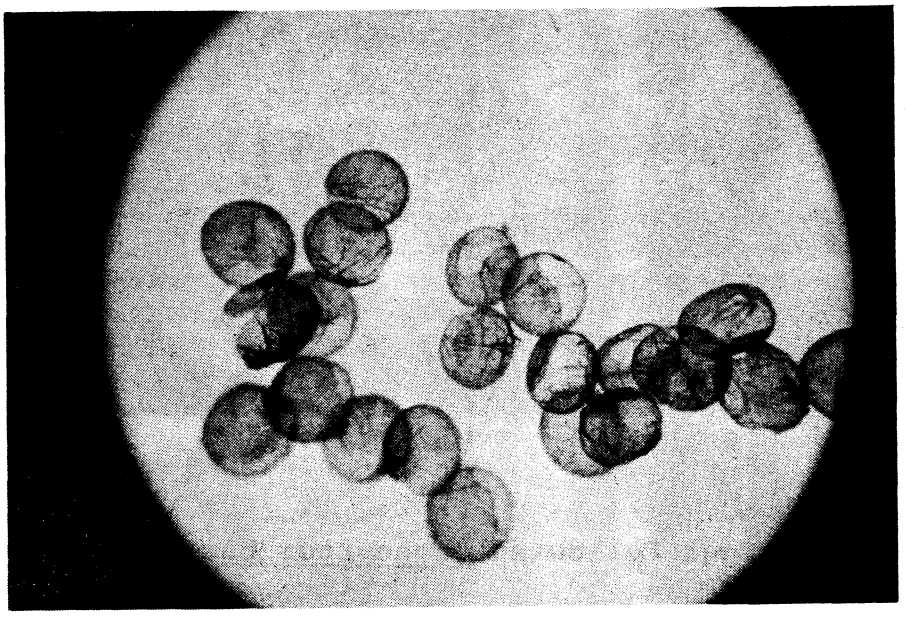
After using the described apparatus on 2,500 gypsy moth egg masses, we conclude that:

1. The use of a vacuum cleaner and home-made screen device provided a rapid, thorough, and accurate method for "de-hairing" and sorting of gypsy moth eggs into groups of viable and "other" eggs.

2. The vacuum pump-pipette apparatus provided a rapid, accurate method of counting gypsy moth eggs. It also simplified the handling of eggs as well as providing a way to further separate eggs from debris.

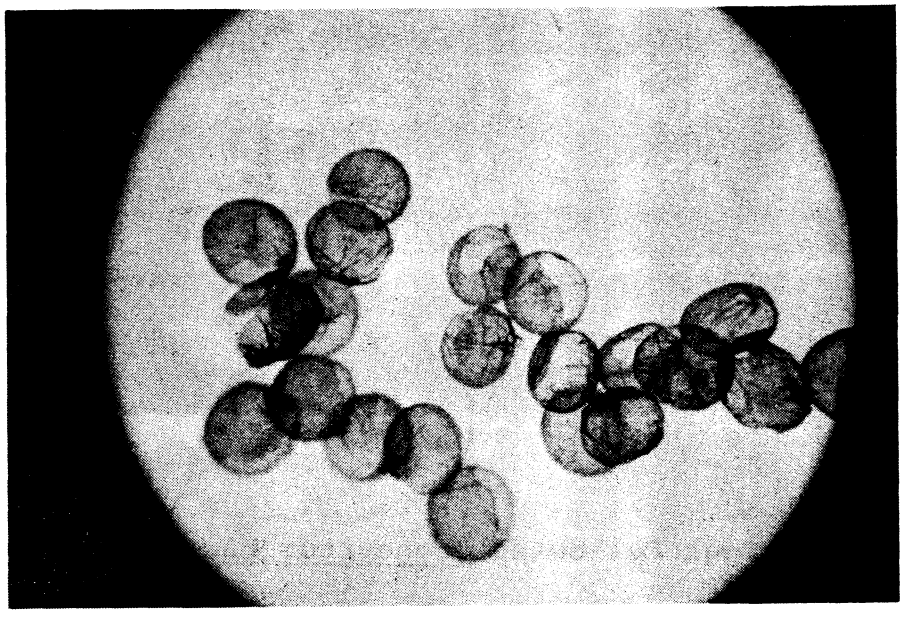
3. Some humidity is necessary in the processing room because static electricity may accumulate on the surface of the eggs causing problems that interfere with the transferring and counting of the eggs.

The ocular inspection method was slow and tedious; but it was found to be an accurate way of separating non-viable eggs on the basis of parasitism, hatch, and non-viability.

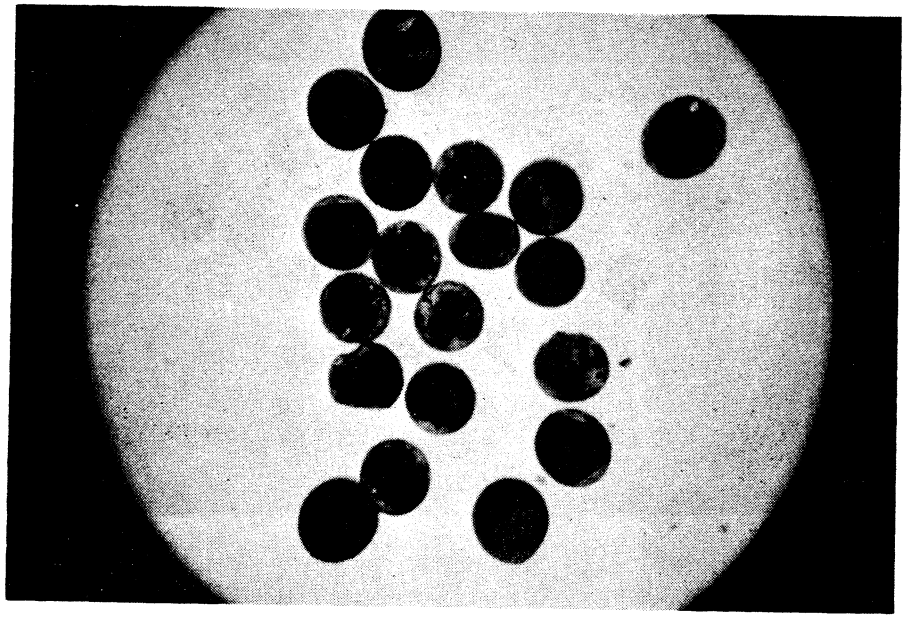


Photograph 8
Gypsy Moth Eggs After Hatch

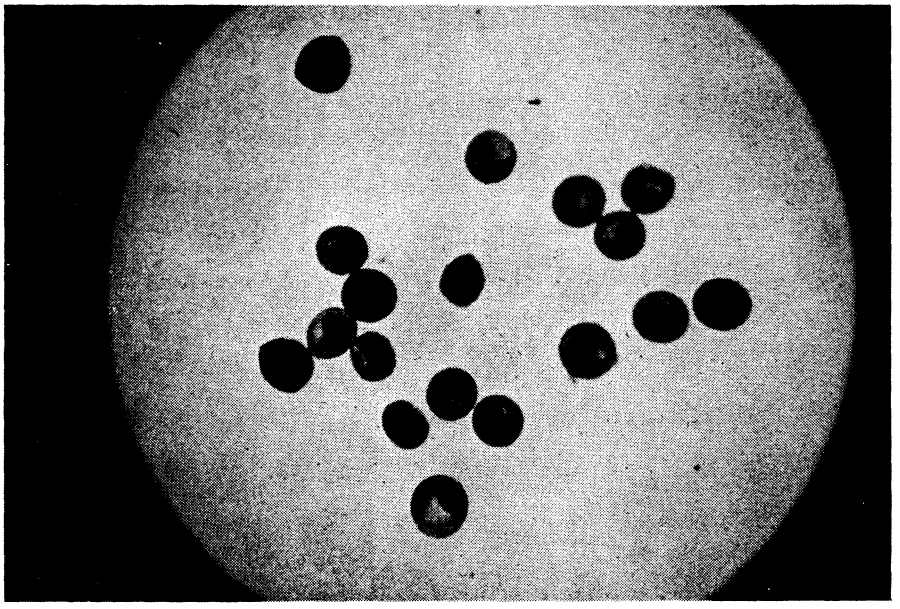
The ocular inspection method was slow and tedious; but it was found to be an accurate way of separating non-viable eggs on the basis of parasitism, hatch, and non-viability.



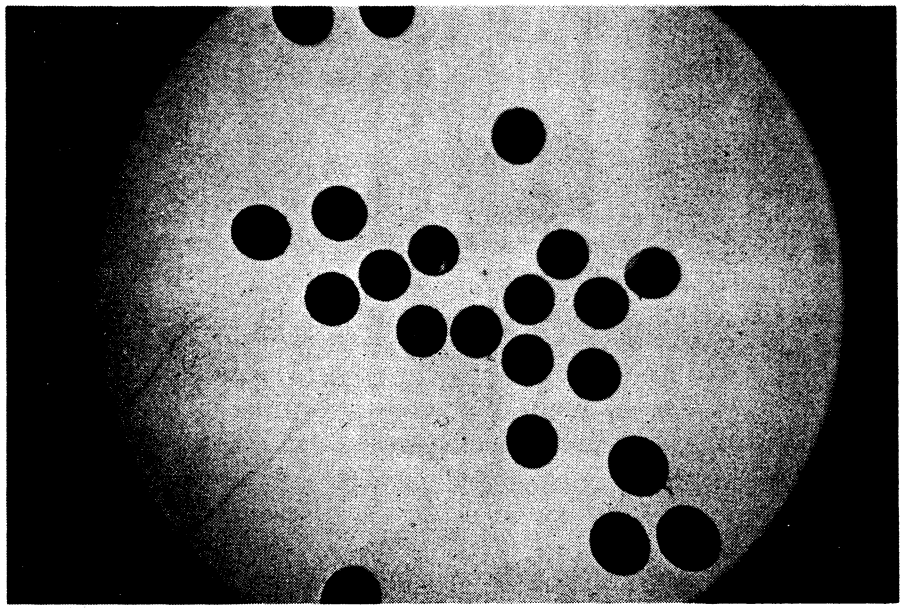
Photograph 8
Gypsy Moth Eggs After Hatch



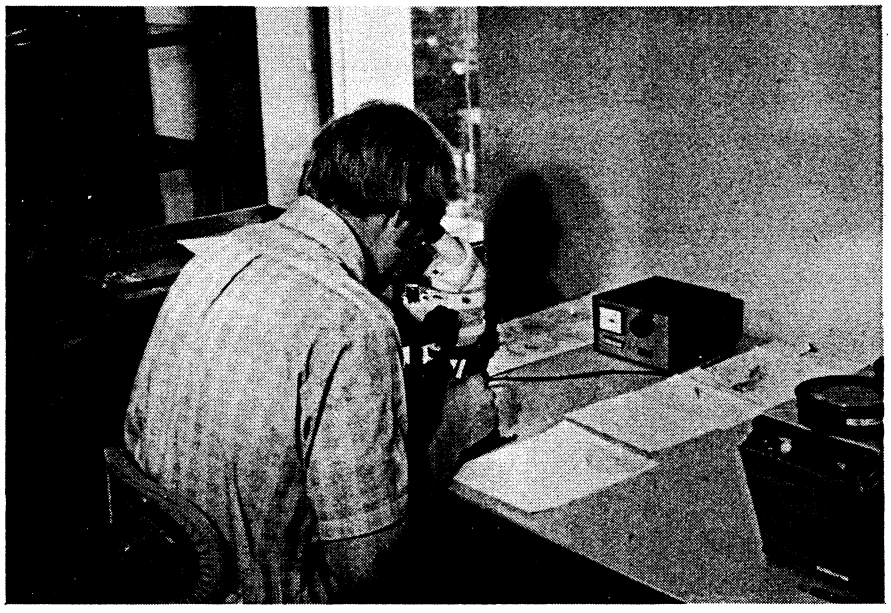
Photograph 10
Gypsy Moth Eggs Containing Dead Ooencyrtus kuwanai



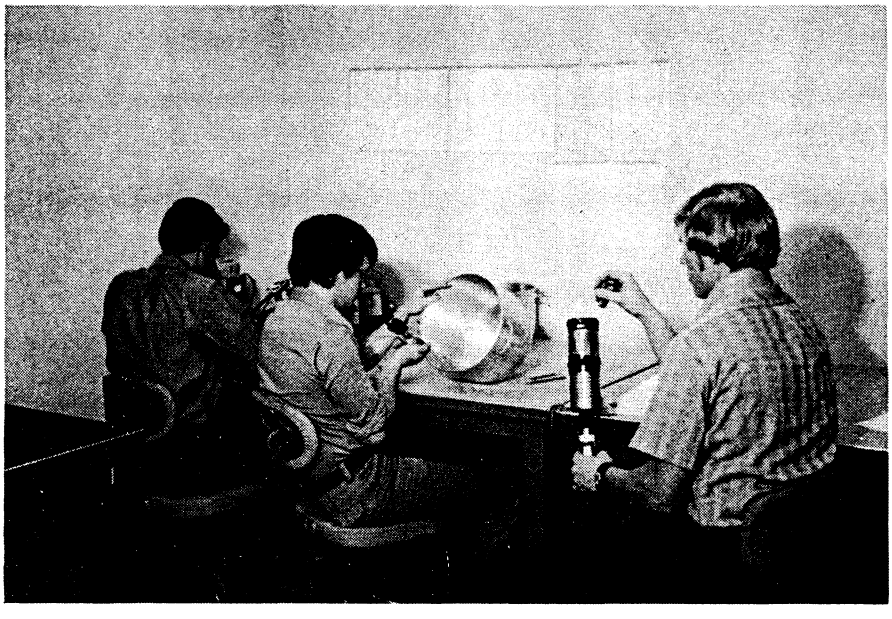
Photograph 11
Aborted Gypsy Moth Eggs



Photograph 12
Viable Gypsy Moth Eggs



Photograph 13
Forestry aid separating gypsy moth eggs with the aid of a binocular microscope.



Photograph 14

Left to right, Forestry Aids, Gary Hare, John Magoon, and Bruce Anderson processing gypsy moth eggs using apparatus described in this report.

APPENDIX D

GYPSY MOTH EGG MASS DATA

Plot Number: Egg Mass Number:___

State : Collection Date:___

County :

Township :

Sprayed :____ Unsprayed: ____

**Block size: Small____ Medium ____ Large ____**

Isolated_____ Adjacent_____

Egg Mass size: Length___mm. width___ mm. Produce___ $mm^2$___

Selected for Weight and Diameter: Yes___ No.___

Number of Eggs: Total_____

Whole_____

Viable:_____ Parasitized_____

Blank:_____

Parasite Emergence: In Days 1___2___3___4___5___6___7___

8___9___10___11___12___13___14___

Parasites: Ooencyrtus Kuwanai Number_____

Other
Parasites_____ _____ Number_____

Other
Parasites_____ _____ Number_____

---

Weight:_____ m.g. (W.O. hair) includes all eggs in the mass)

Diameter: Number in Sieve 10____Sieve12___ Sieve 18___Sieve 20___
(includes viable and blank eggs)

## APPENDIX A

### Gypsy Moth Eggs
### Number to Volume Ratio
### in 1/10 ML. Tubes

### VIABLE EGGS

| ML | No. Eggs in Tube | ML | No. Eggs in Tube |
|---|---|---|---|
| .01 | 7.7 | .27 | 207.9 |
| .02 | 15.4 | .28 | 215.6 |
| .03 | 23.1 | .29 | 223.3 |
| .04 | 30.8 | .30 | 231.0 |
| .05 | 38.5 | .31 | 238.7 |
| .06 | 46.2 | .32 | 246.4 |
| .07 | 53.9 | .33 | 254.1 |
| .08 | 61.6 | .34 | 261.8 |
| .09 | 69.3 | .35 | 269.5 |
| .10 | 77.0 | .36 | 277.2 |
| .11 | 84.7 | .37 | 284.9 |
| .12 | 92.4 | .38 | 292.6 |
| .13 | 100.1 | .39 | 300.3 |
| .14 | 107.8 | .40 | 308.0 |
| .15 | 115.5 | .41 | 315.7 |
| .16 | 123.2 | .42 | 323.4 |
| .17 | 130.9 | .43 | 331.1 |
| .18 | 138.6 | .44 | 338.8 |
| .19 | 146.3 | .45 | 346.5 |
| .20 | 154.0 | .46 | 354.2 |
| .21 | 161.7 | .47 | 361.9 |
| .22 | 169.4 | .48 | 369.6 |
| .23 | 177.1 | .49 | 377.3 |
| .24 | 184.8 | .50 | 385.0 |
| .25 | 192.5 | .51 | 392.7 |
| .26 | 200.2 | .52 | 400.4 |

APPENDIX A - CONTINUED

| ML | No. Eggs in Tube | ML | No. Eggs in Tube |
|---|---|---|---|
| .53 | 408.1 | .78 | 600.6 |
| .54 | 415.8 | .79 | 608.3 |
| .55 | 423.5 | .80 | 616.0 |
| .56 | 431.2 | .81 | 623.7 |
| .57 | 438.9 | .82 | 631.4 |
| .58 | 446.6 | .83 | 639.1 |
| .59 | 454.3 | .84 | 645.8 |
| .60 | 462.0 | .85 | 654.5 |
| .61 | 469.7 | .86 | 662.2 |
| .62 | 477.4 | .87 | 669.9 |
| .63 | 485.1 | .88 | 677.6 |
| .64 | 492.8 | .89 | 685.3 |
| .65 | 500.5 | .90 | 693.0 |
| .66 | 508.2 | .91 | 700.7 |
| .67 | 515.9 | .92 | 708.3 |
| .68 | 523.6 | .93 | 716.1 |
| .69 | 531.3 | .94 | 723.8 |
| .70 | 539.0 | .95 | 731.5 |
| .71 | 546.7 | | |
| .72 | 554.4 | | |
| .73 | 562.1 | | |
| .74 | 569.8 | | |
| .75 | 577.5 | | |
| .76 | 585.2 | | |
| .77 | 592.9 | | |

## APPENDIX B

### Gypsy Moth Eggs
### Number to Volume Ratio
### in 1/10 ML. Tubes

### HOLLOW EGGS

| ML | No. Eggs in Tube | ML | No. Eggs in Tube |
|---|---|---|---|
| .01 | 7.57 | .26 | 196.82 |
| .02 | 15.14 | .27 | 204.39 |
| .03 | 22.71 | .28 | 211.96 |
| .04 | 30.28 | .29 | 219.53 |
| .05 | 37.50 | .30 | 227.10 |
| .06 | 45.42 | .31 | 234.67 |
| .07 | 52.99 | .32 | 242.24 |
| .08 | 60.56 | .33 | 249.81 |
| .09 | 68.13 | .34 | 257.38 |
| .10 | 75.70 | .35 | 264.95 |
| .11 | 83.27 | .36 | 272.52 |
| .12 | 90.84 | .37 | 280.09 |
| .13 | 98.41 | .38 | 287.66 |
| .14 | 105.98 | .39 | 295.23 |
| .15 | 113.55 | .40 | 302.80 |
| .16 | 121.12 | .41 | 310.37 |
| .17 | 128.69 | .42 | 317.94 |
| .18 | 136.26 | .43 | 325.51 |
| .19 | 143.83 | .44 | 333.08 |
| .20 | 151.40 | .45 | 340.65 |
| .21 | 158.97 | .46 | 348.22 |
| .22 | 166.54 | .47 | 355.79 |
| .23 | 174.11 | .48 | 363.36 |
| .24 | 181.68 | .49 | 370.93 |
| .25 | 189.25 | .50 | 378.50 |

APPENDIX B - CONTINUED

| ML | No. Eggs in Tube | ML | No. Eggs in Tube |
|---|---|---|---|
| .51 | 386.07 | .76 | 575.32 |
| .52 | 393.64 | .77 | 582.89 |
| .53 | 401.21 | .78 | 590.46 |
| .54 | 408.78 | .79 | 598.03 |
| .55 | 416.35 | .80 | 605.60 |
| .56 | 423.92 | .81 | 613.17 |
| .57 | 431.49 | .82 | 620.74 |
| .58 | 439.06 | .83 | 628.31 |
| .59 | 446.63 | .84 | 635.88 |
| .60 | 454.20 | .85 | 634.45 |
| .61 | 461.77 | .86 | 651.02 |
| .62 | 469.34 | .87 | 658.59 |
| .63 | 476.91 | .88 | 666.16 |
| .64 | 484.48 | .89 | 673.73 |
| .65 | 492.05 | .90 | 681.30 |
| .66 | 499.62 | .91 | 688.87 |
| .67 | 507.19 | .92 | 696.44 |
| .68 | 514.76 | .93 | 704.01 |
| .69 | 522.33 | .94 | 711.58 |
| .70 | 529.90 | .95 | 719.15 |
| .71 | 537.47 | | |
| .72 | 545.04 | | |
| .73 | 552.61 | | |
| .74 | 560.18 | | |
| .75 | 567.75 | | |

## APPENDIX C

Paired t test for significant difference between hand counted and volumetrically counted gypsy moth eggs

| Tube Sample $A_1$ | Hand Samples $B_1$ | $d=A_1-B_1$ | $d^2$ |
|---|---|---|---|
| 358 | 368 | +10 | 100 |
| 100 | 109 | + 9 | 81 |
| 85 | 85 | + 0 | 0 |
| 43 | 43 | + 0 | 0 |
| 366 | 359 | - 7 | 49 |
| 85 | 82 | - 3 | 9 |
| 66 | 65 | - 1 | 1 |
| 278 | 255 | -23 | 1058 |
| 31 | 27 | - 4 | 8 |
| 134 | 141 | + 7 | 49 |
| 207 | 195 | -12 | 144 |
| 77 | 63 | -14 | 196 |
| 546 | 466 | -80 | 6400 |
| 108 | 105 | - 3 | 9 |
| 139 | 157 | +18 | 324 |
| 115 | 112 | - 3 | 9 |
| 505 | 516 | +11 | 121 |
| 136 | 150 | +14 | 196 |
| 92 | 84 | - 8 | 64 |
| 116 | 110 | - 6 | 36 |
| 82 | 81 | - 1 | 1 |
| 38 | 46 | + 8 | 64 |
| 172 | 177 | + 5 | 25 |

APPENDIX C - CONTINUED

| | | | |
|---|---|---|---|
| 124 | 120 | - 4 | 16 |
| 255 | 218 | +37 | 1369 |
| 30 | 30 | + 0 | 0 |
| | | SUM -50 | 9807 |

$\bar{x}$ = 64.9 and 60.2 Calculated Value of $\underline{t}$ =1.191

Table value of $\underline{t}$ 95% =2.064

APPENDIX E CONTINUED

| MACHINE SORTED | HAND SORTED | $d_2 - d_1$ | $Chi^2$ |
|---|---|---|---|
| 62 | 61 | + 1 | .016 |
| 146 | 145 | + 1 | .006 |
| 108 | 106 | + 2 | .037 |
| 123 | 125 | - 2 | .032 |
| 38 | 40 | - 2 | .100 |
| 592 | 615 | -23 | .860 |
| 261 | 264 | - 3 | .035 |
| 69 | 69 | 0 | 0 |
| 146 | 154 | - 8 | .415 |
| 77 | 83 | - 6 | .433 |
| 131 | 131 | 0 | 0 |
| 184 | 185 | - 1 | .005 |
| 262 | 262 | 0 | 0 |
| 462 | 462 | 0 | 0 |
| 932 | 932 | 0 | 0 |
| 77 | 75 | - 2 | .053 |
| 239 | 239 | 0 | 0 |
| 424 | 422 | + 2 | .009 |
| 30 | 30 | 0 | 0 |
| 254 | 255 | - 1 | .003 |
| 100 | 102 | - 2 | .039 |
| 100 | 99 | - 1 | .010 |
| 130 | 129 | + 1 | .007 |
| 130 | 129 | + 1 | 0 |
| 185 | 187 | - 2 | .021 |
| 0 | 0 | 0 | 0 |
| 46 | 46 | 0 | 0 |
| 177 | 177 | 0 | 0 |
| 124 | 124 | 0 | 0 |

APPENDIX D CONTINUED

| MACHINE SORTED | HAND SORTED | $d_2 - d_1$ | $Chi^2$ |
|---|---|---|---|
| 161 | 161 | 0 | 0 |
| 769 | 769 | 0 | 0 |
| 515 | 515 | 0 | 0 |
| 416 | 416 | 0 | 0 |
| 238 | 238 | 0 | 0 |
| 484 | 488 | - 4 | .032 |
| 385 | 385 | 0 | 0 |
| 516 | 519 | - 3 | .017 |
| 123 | 123 | 0 | 0 |

$x^2 = 9.856$

Table $x^2$ .05 = 113.14

APPENDIX E D

CHI $^2$ TEST FOR SIGNIFICANT DIFFERENCE BETWEEN VACUUM SORTED AND HAND SORTED GYPSY MOTH EGGS

| MACHINE SORTED | HAND SORTED | $d_2 - d_1$ | $Chi^2$ |
|---|---|---|---|
| 277 | 291 | -14 | .673 |
| 177 | 177 | 0 | 0 |
| 184 | 203 | -19 | 1.778 |
| 77 | 83 | - 6 | .433 |
| 123 | 130 | - 7 | .376 |
| 535 | 543 | - 8 | .117 |
| 131 | 135 | - 4 | .118 |
| 123 | 123 | 0 | 0 |
| 92 | 93 | - 1 | .010 |
| 89 | 96 | - 7 | .526 |
| 54 | 58 | 0 | 0 |
| 92 | 92 | 0 | 0 |
| 184 | 189 | - 5 | .270 |
| 855 | 857 | - 2 | .004 |
| 92 | 92 | 0 | 0 |
| 266 | 266 | 0 | 0 |
| 261 | 264 | - 3 | .034 |
| 108 | 109 | 0 | 0 |
| 115 | 115 | - 1 | .008 |
| 39 | 41 | - 2 | .097 |
| 85 | 85 | 0 | 0 |
| 324 | 324 | 0 | 0 |
| 149 | 155 | - 6 | .232 |

APPENDIX D CONTINUED

| MACHINE SORTED | HAND SORTED | $d_2 - d_1$ | $Chi^2$ |
|---|---|---|---|
| 254 | 267 | -13 | .631 |
| 64 | 65 | - 1 | .015 |
| 524 | 526 | - 2 | .001 |
| 489 | 492 | - 3 | .018 |
| 181 | 187 | - 6 | .192 |
| 758 | 760 | - 2 | .005 |
| 115 | 116 | - 1 | .008 |
| 768 | 770 | - 2 | .005 |
| 131 | 131 | 0 | 0 |
| 111 | 116 | - 5 | .215 |
| 41 | 49 | - 8 | 1.300 |
| 69 | 69 | 0 | 0 |
| 724 | 724 | 0 | 0 |
| 69 | 74 | - 5 | .337 |
| 501 | 505 | - 4 | .031 |
| 334 | 335 | - 1 | .002 |
| 231 | 230 | 1 | .004 |
| 589 | 588 | - 1 | .007 |
| 285 | 285 | 0 | 0 |
| 386 | 386 | 0 | 0 |
| 131 | 132 | - 1 | .007 |
| 103 | 105 | - 2 | .038 |
| 465 | 465 | - 0 | 0 |
| 310 | 310 | 0 | 0 |
| 34 | 34 | 0 | 0 |
| 17 | 19 | - 2 | .210 |
| 262 | 261 | + 1 | .003 |
| 116 | 117 | - 1 | .008 |